OPTIMIZATION OF ENCAPSULATION OF NATURAL DYE BY CYCLODEXTRIN USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Nowadays, cyclodextrin widely used in industries due to its ability in encapsulation process with natural dye. However, the production cost for cyclodextrin is very expensive. A study on how to reduce the production cost by optimization of pH, concentration of cyclodextrin and temperature effect on encapsulation of natural dye by cyclodextrin using Response Surface Methodology (RSM) was successfully done. During the preliminary experiment, one factor at a time was employed to screen the best range for pH, concentration of cyclodextrin and temperature. All the parameter ranges obtained were used in RSM. RSM software in Design Expert version 6.0.8 was used with Central Composite Design (CCD) mode. Seventeen sets of experiments with different parameter values were suggested by the software. The predicted optimum values for pH, concentration of cyclodextrin, temperature and concentration of encapsulation of natural dye-β-cyclodextrin were pH 3.11, 0.004 mol/L, 60 ^{o}C and 1.27763 g/L respectively. One set of experiment was run using the optimized parameter and as a result, 1.2534 g/L concentration on encapsulation of natural dye-β-cyclodextrin was recorded. Before optimization, concentration on encapsulation of natural dye-β-cyclodextrin was only 0.775 g/L and the concentration was increased by 38.17% after optimization. The optimization also reduces the cost and energy consumption as the pH reduced from pH 10.5 to pH 3.11, concentration of cyclodextrin reduced from 0.005 mol/L to 0.004 mol/L and temperature was reduced from 70 ^{o}C to 60 ^{o}C. As a conclusion, this study was successful to increase encapsulation of natural dye by cyclodextrin production, reduce the energy consumption and also be able to reduce the production cost of cyclodextrin.

ABSTRAK

Pada masa kini, siklodekstrin telah digunakan secara meluas dalam industri atas kebolehannya dalam proses pengkapsulan dengan pewarna semulajadi. Walaubagaimanapun, kos penghasilan siklodekstrin sangat mahal. Satu kajian tentang bagaimana mengurangkan kos pengeluaran dengan pengoptimuman pH, kepekatan siklodekstrin dan suhu terhadap pengkapsulan pewarna semulajadi dengan siklodekstrin menggunakan Kaedah Permukaan Tindak balas (RSM) telah berjaya dilakukan. Di awal peringkat eksperimen, kaedah satu faktor pada satu masa telah digunakan untuk saringan julat terbaik untuk pH, kepekatan siklodekstrin dan suhu. Kesemua julat parameter yang diperolehi digunakan dalam RSM. Perisian RSM dalam Design Expert versi 6.0.8 telah digunakan dengan mod Rekabentuk Komposit Pusat (CCD). Tujuh belas set eksperimen berlainan nilai parameter telah dicadangkan oleh perisian ini. Nilai optimum yang diramalkan untuk pH, kepekatan siklodekstrin, suhu dan kepekatan pengkapsulan terhadap pewarna semulajadi dengan siklodekstrin masing-masing pH 3.11, 0.004 mol/L, 60 ^{o}C dan 1.27763 g/L. Satu set eksperimen telah dijalankan bagi menguji parameter yang telah dioptimumkan dan sebagai keputusannya, 1.2534 g/L kepekatan pengkapsulan terhadap pewarna semulajadi dengan siklodekstrin telah direkodkan. Sebelum pengoptimuman, kepekatan pengkapsulan terhadap pewarna semulajadi dengan siklodekstrin hanya 0.775 g/L dan kepekatannya meningkat 38.17%. Pengoptimuman juga mengurangkan kos dan penggunaan tenaga seperti pH dikurangkan dari pH 10.5 kepada pH 3.11, kepekatan siklodekstrin dikurangkan dari 0.005 mol/L kepada 0.004 mol/L dan suhu telah direndahkan dari 70 ^{o}C kepada 60 ^{o}C. Sebagai kesimpulan, kajian ini telah berjaya meningkatkan penghasilan pengkapsulan pewarna semulajadi oleh siklodekstrin, mengurangkan penggunaan tenaga dan juga mampu mengurangkan kos pengeluaran siklodekstrin.

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LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of variance
CCD	-	Central composite design
g	-	Gram
mL	-	Mililitre
g/L	-	Gram per liter
mol/L	-	Molar per liter
L	-	Liter
M	-	Molar (mol/L)
mg	-	Milligram
mM	-	Milimolar
MW	-	Molecular weight
OD_{315}	-	Optical density at 315nm
OFAT	-	One Factor at a Time
RSM	-	Response surface Methodology
rpm	-	Revolution per minutes
T	-	Temperature
U/mg	-	Unit enzyme per miligram
^{o}C	-	Degree Celcius
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cyclodextrins are among of the most remarkable monocyclic molecules, with significant theoretical and practical impacts in chemistry. Cyclodextrins are able to form host-guest complexes with hydrophobic molecules given the unique nature imparted by their structure. As a result, these molecules have found a number of applications in a wide range of fields and widely used in chemical, pharmaceutical, food and other technologies as enzymes mimic, enantioselective catalysts, drug carriers and odor tastes-masking compounds (Chankvetade *et al*., 1993).

Lei and Qing (2002) claims that taking advantage of their inclusion capacity, cyclodextrins are being used in various fields to improve stability of guest substances, example to protect any components that are unstable when exposed to light or oxygen, to prevent volatile components evaporating, to modify physicochemical properties or to eliminate unpleasant tastes or odor.

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In addition, cyclodextrins are also utilized to control release of a fragrance or drug and the non-toxic character of it together with their applicability in pharmaceutical, food and cosmetic sciences is also important for their laboratory and environmentally friendly analytical applications (Lei and Qing, 2002). Therefore, natural dye are employed as carriers for biologically active substances, enzyme models, separating agents, catalysts, mass transfer promoters, additives in perfumes, cosmetics, aliments or food , environmental protection agents or sensors for organic molecules. Cyclodextrin are essentially inert to photochemical excitation but their chemical modification with chromophoric moities may associate spectroscopic properties to the inclusion of guest molecule. For this research, *curcuma longa* is used as the source of extraction in encapsulation of natural dye by cyclodextrins in order to get and maintain the color (Min *et al.*, 2009).

Importance of natural dyes is more relevant world wide in the context of increasing environment consciousness. The non-toxic, biodegradable and eco-friendly properties make them exceedingly popular amongst nature loving and health awarded people. In spite of all, the present international consumption natural dyes are about 1% of the synthetic dye consumption. It is all due to the existing limitations and technical drawbacks of these dyes, which restrict their usage in textile dyeing. Some of these are color yield, complexibility of dyeing process, limited shades, reproducible results, blending problems and inadequate fastness properties. Lack of standardized profiles for extraction and textile dyeing are also major constraints (Kiran and Kapoor, 2007).

Indian craftsmen had monopoly on natural dyes before the advent of synthetic dyes but the craft of natural dyeing was very secretive amongst the particular communities. They did not disclose their trade secrets to any one except their family members. These techniques varied a lot depending upon the communities practicing in the different regions of India. They had no communication system and no documentation was made. In the era of synthetic dyes, natural dyeing became uncompetitive with regards to limited shades, less fastness properties and higher cost and thus neglected. It forced the craftsmen to switch over to other professions

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thereby causing major set back to natural dyeing and the traditional knowledge became abdomened. Technical drawbacks and limitation of natural dyes can be solved through adequate scientific.

1.2 Problem Statement

Synthetic dyes have their own worth. Synthetic dyes for example hair dyes cannot maintain the color in long time compare to the natural dyes. It was reported that, the resulting dry of natural dye concentrated maintained its stability after sixteen and one half weeks of storage in dark and having good color. Natural dyes also give glow to skin and keep some harmful bacteria away from the body. Presently, the yellow pigments, turmeric and tartrazine are approved by the Food and Drug Administration (FDA) for use in foods and beverages. Tartrazine is a stable and water soluble synthetic dye. Turmeric, on the other hand is water insoluble and relatively unstable.

However, turmeric is a natural rather than a synthetic dye. Natural dyes have been tested and was reported that, it is hard to be absorbed and hard to be color on the clothes. So, the type of cyclodextrins has been used in this experiment because of their characteristics as the water soluble dyes in water based systems for natural dye. Cyclodextrin inclusion is a molecular phenomenon in which usually only the guest molecule interacts with the cavity of a cyclodextrin molecule to become entrapped and form a stable association. Molecules or functional groups of molecules those are less hydrophilic than water can be included in the cyclodextrin cavity in the presence of water. So, the molecules of natural dye solution can be fit, at least partly into cyclodextrin cavity and can help the coloring to be part of the cloth.

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1.3 Objectives

The objective of this research is to optimize the encapsulation of natural dye by cyclodextrin using response surface methodology (RSM).

1.4 Scopes of Study

To achieve the objectives, scopes has been identified in this research. The scopes of this research are listed as below:

a) To compare the effect of cyclodextrin types in encapsulation of natural dye
b) To study the effect of pH, concentration and temperature on encapsulation of natural dye by cyclodextrin
c) To optimize the concentration, temperature and pH on encapsulation of natural dye by cyclodextrin using response surface methodology (RSM)

1.5 Significance of Study

Potential markets for natural dye by cyclodextrins in future are expected to be huge by researchers, although the current market is not so large and development efforts, exploring various exploring end uses, are currently going on worldwide.

Advantage of the research is that it provides a new, useful and unique product that should have a high market demand. The production of natural dye by cyclodextrins should be improved and developed because of the profitable for the economy. Therefore, findings the way to produce higher amount and quality of natural dye by cyclodextrins and their derivatives is important because of the expected consumption for the future (Lei and Qing, 2002).

It was reported that, the resulting dry of natural dye concentrated maintained its stability after sixteen and one half weeks of storage in dark and having a good color. Natural dye also can be fit and maintain the colour on the cloth in long time because of their characteristic as water soluble dyes in water based systems for natural dye.

CHAPTER 2

LITERATURE RIVIEW

2.1 Natural Dye

Today dyeing is important and popular amongst natural loving and health awarded people. Nearly all dyes is currently produced from synthetic compounds. This means that costs have been greatly reduced and certain applications far enhanced. Customers more aware of environmental issues are now demanding natural products, naturally sourced. If a fashion company introduces a new line of clothes produced with a natural fibre, the naturally sourced dye is needed to complete the green label. Natural dyes can offer not only a rich and varied source of dyes, but also the possibility of an income through sustainable harvest of the dye plants. They also have a far superior aesthetic quality, which is much more pleasing to the eye.

Nature provides a wealth of plants which will yield their colours for the purpose of dyeing. Most of the natural dyes are adjective in nature. They require a suitable fixing agent under dyeing process. There is a lot of possibilities to obtain numerous shades with good fastness properties by using suitable safe mordant. Natural dye is gaining popularity especially in the food, cloth and beverage sectors due to strong demand for more natural products by health-conscious consumers (Rebecca *et al*., 2008). Some examples of natural colorants which have already been used in the textile industry include anthocyanin, lycopene, paprika extract and curcumin.

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In addition, natural colours and pigment from fruits and vegetables may contribute additional nutritional value to food coloured as observed in cactus fruits (Mohammer *et al.,* 2005). For this research, turmeric is used as the source of extraction in encapsulation of natural dye by clodextrins in order to get and maintain the color.

2.1.1 Turmeric

The experiment deals with standardization of extraction and dyeing profiles for traditionally known dye from the rhizomes of turmeric (*curcuma longa*). Turmeric (*curcuma longa*) is an importance spice used as a cosmetic and coloring agent has also been used Indigenous System of Medicine. Turmeric is mainly valued for its principle coloring constituent curcumin, which imparts the yellow color on textile fibers and food items. Rhizome, the main source of curcumin also contains various ingredients like protein, fat, fibers, carbohydrates, essential oil and others. Curcumin processes various bioactive properties and is used in modern system of medicine. It is well recognized for its anti-flammatory, hepatoprotective, anticancer, metabolic disorders, antimicrobial, antiviral and antioxidant activities (Herbach *et al.,* 2007).

The yellow pigmented fraction curcumin is isolated from the rhizomes of *curcuma longa* rhizomes are also used for diverse medicinal or food purposes. Turmeric is closely related to ginger and the methods by which ginger oleoresin is obtained may be applied to turmeric, the curcumin and the volatile-oil being both extracted by the same volatile-solvent. Until the late 1970s, turmeric oleoresin was prepared only in a few of the consuming countries, mainly in United States as well in the United Kingdom and manufacturers refuse to disclose their methods of processing. India, however, has now embarked upon commercial production of turmeric oleoresin following the investigation of and the publication of information on processing techniques by the Central Food Technological Research Institute in Mysore (Rohe *et al.,* 2006).

The food colour curcumin (turmeric yellow) is obtained by solvent extraction of turmeric which is the ground rhizomes of *curcuma longa*., with purification of the resultant extract by crystallization. The commercial product consists essentially of curcumins: the colouring principle (1,7-*bis* (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) and its desmethoxy and bisdesmethoxy derivatives in varying proportions. The total content of colouring matter (*curcuminoids*) in *curcumin* is not less than 90%. The term "*curcumin*" in this monograph refers to the material for which specifications exist. The principal colouring component, 1,7-*bis* (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione, is often referred to as *curcumin* in the literature. To avoid confusion, the researcher decided that this report would not use the term of *curcumin* when referring to this substance. A common synonym for this substance is diferuloylmethane and this name will be used when it is necessary to refer to the principal colouring component of *curcumin* (Benford, 2001).

2.1.2 Dragon Fruit

Fruits of the genus *Hylocereus* (Berger) Briton and rose originated from Latin America and are known as red pitaya belonging to the *Cactaceae* family (Kugler *et al.,* 2007). This vine-like epiphytic cactus is also cultivated in Vietnam, Malaysia, Taiwan, China, Okinawa, Israel and Southern China. Producing a deep purple-coloured flesh comparable to red beet or amaranth, fruits from *Hylocereus polyrhizus* are highly appealing in the European and United States market.

Dragon fruit is one of the new focus for the next source of natural dye because it is rich in betalains which are the similar array of colour pigments found in beetroot. Beetroot has been the most important betalain source for natural red colouring and is mainly composed of the red-purple betanin and the C15-isomer isobetanin. However, there is a demand for alternative compounds because of the unfavourable earthy flavour caused by geosmin and pyrazine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines (Esquivel *et al.,* 2007).

Hence, fruits from the *Cactaceae* family have been suggested as a promising betalain source being devoid of the mentioned drawbacks. Betalains are nitrogenous vacuolar pigments and important chemotaxonomical markers found in 13 families within the plant kingdom and in some members of the Basidiomycetes (Kugler *et al.,* 2007). Betalains have never been found co-existing with the widely distributed plant pigment anthocyanon which explains their roles as markers.

Some advantages that betalains possess over anthocyanins include being more water soluble, a tinctorial strength up to three times higher than anthocyanins and a wider pH stability range from pH 3 to 7 making it suitable for application in a broad palette of low-acid and neutral food (Diego *et al*., 2008). Betalains are divided into the red-purple betacyanins and yellow-orange betaxanthins which comprise about 55 different structures and promise a great variation of colour array to the food industry. In dragon fruit alone, there are at least seven known betalain namely; betanin, isobetanin, phyllocactin, isophyllocactin, betanidin, isobetanidin and bougainvillein-r-I (Sadilova *et al.,* 2006) all of which have identical absorption spectra (AmaK) that contribute to the deep-purple colour observed in the fruit pulp.

2.2 Cyclodextrin

Cyclodextrin inclusion is a molecular phenomenon in which usually only one guest molecule interacts with the cavity of a cyclodextrin molecule to become entrapped and form a stable association. Molecules or functional groups of molecules those are less hydrophilic than water, can be included in the cyclodextrin cavity in the presence of water (Min *et al*., 2009).

In order to become complex, the "guest molecules" should fit, at least partly, into the cyclodextrin cavity. The cavity sizes as well as possible chemical modifications determine the affinity of cyclodextrins to the various molecules. In the case of some low molecular weight molecules, more than one guest molecule may fit into the cavity. On the opposite, some high molecular weight molecules may bind more than one cyclodextrin molecule (Min *et al*., 2009).

Cyclodextrins, as known today is called cellulosine. The history of the three naturally occurring cyclodextrins a, ß and γ has been identified by Schardinger, (1935). These compounds were therefore referred to as "schardinger sugars". For 25 years, between 1911 and 1935, Pringsheim in Germany was the leading researcher in this area, demonstrating that cyclodextrins formed stable aqueous complexes with many other chemicals. By the mid 1970's, each of the natural cyclodextrins had been structurally and chemically characterized and many more complexes had been studied. Others, usually smaller molecules (called guests) can enter their cavity forming inclusion complexes with these hosts. Since the 1970s, extensive work has been conducted by Szejtli (scientist) in 1970 and others exploring encapsulation by cyclodextrins and their derivatives for industrial and pharmacologic applications (Lajos *et al*., 1998).

Typical cyclodextrins are constituted by 6-8 glucopyranoside units can be topologically represented as toroids with the larger and the smaller openings of the toroid exposing to the solvent secondary and primary hydroxyl group respectively. Because of this arrangement, the interior of the toroids is not hydrophobic but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic to impart cyclodextrins or their complexes water solubility.

The formation of the inclusion compound greatly modifies the physical and chemical properties of the guest molecule, mostly in terms of water solubility. This is the reason why cyclodextrins have attracted much interest in many fields, especially pharmaceutical applications because inclusion compounds of cyclodextrins with hydrophobic molecules are able to penetrate body tissues. These can be used to release biologically active compounds under specific conditions. In most cases the mechanism of controlled degradation of such complexes is based on pH change of water solutions, leading to the cleavage of hydrogen or ionic bonds between the host and the guest molecules. Alternative means for the disruption of the complexes take advantage of heating or action of enzymes able to cleave α-1,4 linkages between glucose monomers (Dodziuk, 2006).

2.2.1 Advantages of Cyclodextrins

Cyclodextrins are able to form host-guest complexes with hydrophobic molecules given the unique nature imparted by their structure. As a result these molecules have found a number of applications in a wide range of fields. Cyclodextrins are widely applied in pharmaceutical formulations to enhance the solubility, stability and bioavailability of drug molecule (Lei and Qing, 2002).

Cyclodextrins are able to form inclusion complexes with broad range hydrophobic molecules as poorly soluble drugs, rapidly deteriorating flavours,

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volatile fragrances, toxic pesticides or dangerous explosives, even gases, entrapping these substances in their inner cavities. For example, α-cyclodextrin forms inclusion complexes with both aliphatic hydrocarbons and gases, such as carbon dioxide. β-cyclodextrin typically forms complexes with small aromatic molecules. γ-cyclodextrin can accept more bulky compounds, including vitamin D2 or organic macro cycles (Chankvetade *et al*., 1993).

Cyclodextrins are used to maintain flavor and color as well as for the dietary supplements. The amino acids and vitamins can be stabilized and protected by cyclodextrins. The cyclodextrins are used in the cosmetics field to solubilize fragrances, to suppress their volatility and to allow perfume-containing to be sprayed as micropowders. The strong ability of complexing fragrances can also be used for another purpose. First dry, solid cyclodextrin microparticles are exposed to a controlled contact with fumes of active compounds, then they are added to fabric or paper products. Such devices are capable of releasing fragrances during ironing or when heated by human body. Such a device commonly used is a typical 'dryer sheet'. The heat from clothes dryer releases the fragrance into the clothing. Cyclodextrins are also used as stabilizers, emulsifiers and deodorants (Dodziuk, 2006).

In industry, cyclodextrin is used to produce the ink including the water soluble dyes in water based systems for ink jet printers, water resistance-fixing characteristics in ink jet printers, water resistance in thermal ink jet printing, aqueous composition or wettability in writing ustencils and greater florescent yield in highlighter pen (Demchuk *et al*., 2004).

Textile products with specific functions have been developed. Cyclodextrins are essential ingredients for the production of such new materials in the textile field. Cyclodextrins are mainly used for keeping moisture in squalance fibers, reducing odors in fibers containing an added antibacterial agent. The ability of cyclodextrins

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to form complexes with hydrophobic molecules has led to their usage in molecular chemistry (Lei and Qing, 2002).

2.3 Ultraviolet-Visible Spectroscopy

Application of ultraviolet-visible spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Solutions of transition metal ions can be colored (for example, absorb visible light) because the electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue, adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λmax) (Kolbesen and Doll, 2005).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the ultra violet or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, or ethanol for organic-soluble compounds. Organic solvents may have significant ultra violet absorption, not all solvents are suitable for use in ultra violet spectroscopy. Ethanol absorbs very weakly at most wavelengths. Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases. While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement (Kolbesen and Doll, 2005).

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The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, ultra-violet spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from molar extinction coefficients or more accurately, determined from a calibration curve. Figure 1 below show the prism of ultra-violet spectroscopy.

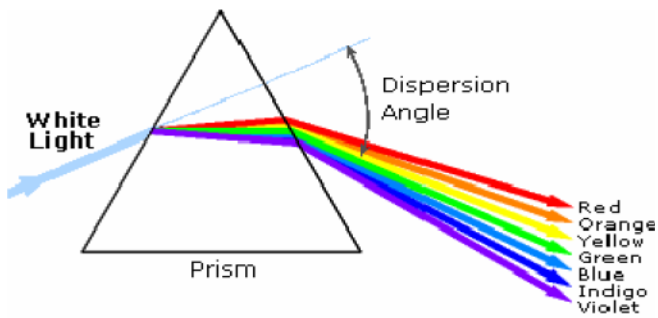

Figure 2.1 : Prism of ultra-violet spectroscopy

Employment of ultra-violet spectroscopy in encapsulation natural dye by cyclodextrins (CDs) production has been reported by Min *et. al*., (2009). From their findings, the formation of complex of natural dye with the types of cyclodextrin in aqueous solution was characterized by ultra-violet spectroscopy. The absorption spectra of natural dye in the absence of cyclodextrin are showed through the absorption peaks. The absorption increasing with the increasing of concentration of cyclodextrin (Min *et al*., 2009).

2.4 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. The most extensive applications of RSM are in the particular situations where several input variables potentially influence some performance measure or quality characteristic of the process. Thus performance measure or quality characteristic is called the response. The input variables are sometimes called independent variables